



ORIGINAL ARTICLE

Caries status and quantification of four bacteria in saliva of Chinese preschool children: A cross-sectional study



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Abstract *Background/purpose:* Mutans streptococci, bifidobacteria, and lactobacilli are acidogenic and aciduric bacteria which may be associated with early childhood caries (ECC). This study aimed to analyze the bacterial composition of four cariogenic microorganisms, namely *Streptococcus mutans*, *Streptococcus sobrinus*, *Bifidobacterium* spp., and *Lactobacillus* spp. in the saliva of Chinese preschool children and to assess the association of the bacterial burden with the caries status.

Materials and methods: A total of 109 saliva samples were collected from 36 children with ECC and 73 caries-free (CF) children aged 2–4 years. A questionnaire was used to evaluate other caries risk factors such as dietary habits and oral hygiene practice. A quantitative real-time polymerase chain reaction was performed to determine the presence and loads of the four microorganisms. Pearson's correlation test was conducted to evaluate the relationship between the World Health Organization caries diagnostic criteria dmft/dmfs index scores and saliva levels of the selected bacteria.

Results: The prevalence of *S. mutans* and *S. sobrinus* in children with ECC was 88.9% and 19.4%, respectively, whereas the prevalence in CF children was 76.7% and 4.1%, respectively. The saliva levels of *S. mutans* and *S. sobrinus* in children with ECC were significantly higher than in CF children ($P = 0.004$ and $P = 0.007$, respectively) and positively correlated with

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dmft/dmfs index scores ($r = 0.321/0.316$ and $r = 0.271/0.245$, respectively). An association was found between children with caries-active lesions and the frequency of sugar consumption ($P = 0.004$). No significant difference in the presence and load of *Bifidobacterium* spp. or *Lactobacillus* spp. were found between the two groups.

Conclusion: *S. mutans*, *S. sobrinus*, and sugar consumption appear to be associated with caries status in the studied population, which might be useful for caries screening in Chinese preschool children.

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Introduction

Early childhood caries (ECC), dental caries in the primary dentition, is a major health problem worldwide and affects 38% of children in the USA and over 40% of Chinese children.^{1,2} For many children, ECC is a source of pain, chewing difficulties, speech problems, psychological problems, and a lowered quality of life. Children experiencing caries in their primary dentition are likely to have more caries later in life, affecting the permanent dentition.³

Despite its public health significance, the etiological factors involved in ECC are still incompletely understood. Several epidemiological studies have shown that mutans streptococci (MS, mostly *Streptococcus mutans* and *Streptococcus sobrinus*) are associated with ECC.^{4,5} A number of studies, however, have reported that the mere presence of *S. mutans* or *S. sobrinus* is not sufficient to predict the occurrence of dental caries in children.^{6,7} In addition, due to their acidogenicity and aciduricity, bifidobacteria⁸ and lactobacilli⁹ have been reported to be associated with ECC by some workers. Bifidobacteria¹⁰ and lactobacilli¹¹ have also been introduced as probiotics in caries prevention. Based on these controversial observations and the fact that MS, bifidobacteria, and lactobacilli are the most frequent caries-associated bacteria,^{8,12} four microorganisms, namely *S. mutans*, *S. sobrinus*, *Bifidobacterium* spp., and *Lactobacillus* spp., were selected as target microorganisms in the present study.

Several studies have qualitatively and quantitatively described the microbiology of dental caries in various populations.^{13–15} However, culture-independent studies describing the amount of distinct microorganisms in the saliva of Chinese preschool children with ECC have not so far been published. This study aimed to analyze the bacterial composition of these four microorganisms in the saliva of Chinese preschool children using a quantitative real-time polymerase chain reaction (qPCR). The study hypothesized that all four targeted microorganisms would be more abundant in the saliva of children with active caries than in caries-free (CF) children, and that the saliva levels of the selected bacteria would be associated with the severity of ECC.

Materials and methods

Participant population

Approval for this study was obtained from the Human Ethics Research Committee of the Hospital of Stomatology, Wuhan

University, Wuhan, China. A total of 109 participants were randomly recruited from two kindergartens in Wuhan, China. Informed consent was obtained from the parents of all the children. The children ranged in age from 24 months to 48 months. Thirty-six children with active caries were diagnosed as having ECC and the remaining 73 children without caries belonged to the CF group. The exclusion criteria included: the use of systemic drugs; the use of systemic antibiotic drugs or an antibacterial mouth rinse 2 months prior to the commencement of the study; or the local application of fluoride or antibacterial agents 3 months prior to start of the study.

Clinical examination and questionnaires

The children were examined clinically in the dental clinic by one calibrated dental epidemiologist. The agreement for intra-examiner results was assessed for assurance of the quality of the data obtained (>0.85). The clinical oral health status was measured using the decayed (d), missing due to caries (m), and filled (f) tooth (t) or surface (s) (dmft or dmfs) World Health Organization caries diagnostic criteria. Data, including oral hygiene practice and frequency of sugar consumption of each child, were collected by questionnaire after the examination.

Sampling and extraction of genomic DNA

Sampling was performed in the morning and all the children were asked to avoid eating or drinking for at least 1 hour prior to oral sampling. Nonstimulated whole saliva samples (at least 2 mL) were collected from the children's mouths around the sublingual and mucosal surfaces with a sterile syringe according to a previously published method.¹⁶

Total bacterial genomic DNA was extracted from each saliva sample using a modification of the Epicentre method (Epicentre, Madison, WI, USA) based on a published protocol.¹⁷ A standard concentration of 10 ng/ μ L was prepared for each individual sample for all qPCR assays.

Bacterial strains

Four oral bacterial strains comprising four species were used in this study for construction of the quantification standard curves. *S. mutans* strain UA159 and *S. sobrinus* strain 6715 were supplied by the China Center for Type Culture Collection (CCTCC, Wuhan, China). *Bifidobacterium dentium* strain ATCC 27534 and *Lactobacillus*

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